

**Remarks**

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 1, 2, 14, 15, 47, 61-63, 67, and 68 are amended, and claims 69-73 are added. The amendments are intended to advance the application and are not intended to concede to the correctness of the Examiner's position or to prejudice the prosecution of the claims prior to amendment, which claims are present in a continuation of the above-identified application. Claims 1-6, 9, 11-12, 14-15, 18, 20-21, 24-39, 41-45, 47, 60-64, and 67-73 are pending.

Amended claims 1, 2, 14-15, 47, and 63 are supported by originally-filed claims 1, 2, 14-15, 47 and 63, respectively.

Amended claim 67 is supported by originally-filed claims 1 and 11.

Amended claim 68 is supported by originally-filed claim 1.

New claim 69 is supported at page 53, lines 19-20 of the specification.

New claim 70 is supported at page 60, lines 18-21 of the specification, and new claim 71 is supported at page 20, lines 4-20 of the specification.

New claim 72 is supported at page 68, lines 21-22 of the specification.

New claim 73 is supported by originally-filed claims 1 and 63.

With respect to claim 64, the Examiner is requested to consider that claim 64 depends on claim 63, which were both assigned to the claims in Group I, the elected group of claims (see the Restriction Requirement dated September 10, 2002 and the Response to the Restriction Requirement dated November 13, 2002).

**The 35 U.S.C. § 112, Second Paragraph, Rejections**

The Examiner rejected claims 1-9, 11-12, 14-15, 18, 20-21, 24-39, 41-45, 47, 60-63, and 67-68 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. This rejection, as it may relate to the pending claims, is respectfully traversed.

In particular, the Examiner asserts that the following phrases are indefinite: "transcription regulatory sequences composition," "a reduced number of transcription factor binding

sequences,” and/or “a reduced number of intron splice sites, poly(A) addition sites and promoter sequences,” as without knowing all of the possible sequences which are considered to be transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences, the number of such sequences could not be calculated.

The phrase “transcription regulatory sequence composition” no longer appears in the claims, rendering the § 112(2) rejection over that phrase moot.

With regard to the other phrases, it is Applicant’s position that “transcription factor binding sequences,” “intron splice sites,” “poly(A) addition sites,” and “promoter sequences” are phrases conventionally used and recognized by the art, as evidenced by Iannacone et al. (Plant Mol. Biol., 34:485 (1997); see Table 1, “polyadenylation sequences” and page 488 “promoter”) Zolotukhin et al. (U.S. Patent No. 5,874,304; “promoters” – see section entitled “Promoters,” and “intron” in Examples I-II and IX), and Sherf et al. (U.S. Patent No. 5,670,356; see “transcription factor binding site” at column 8, line 67), references cited against the claims under 35 U.S.C. § 103(a). Thus, one of ordinary skill in the art prior to Applicant’s filing conventionally used and understood these terms. Yet further evidence for the conventional use of these phrases is provided in the following abstracts: Wahle et al. (FEMS Microbiol. Rev., 23:277 (1999); “poly(A)tail” and “AAUAAA sequence”); Hampsey (Microbiol. Mol. Biol. Rev., 62:465 (1998); “promoter” and “transcription factors”); and Konarska (Acta. Biochim. Pol., 45:869 (1998); “splice site”) (a copy of each is enclosed herewith). Accordingly, the metes and bounds of those terms is definite.

Moreover, the reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences in the synthetic nucleic acid molecule is relative to the number of those sequences in a wild type or parent sequence. Thus, the number is readily calculatable.

Therefore, withdrawal of the § 112(2) rejection is respectfully requested.

*The 35 U.S.C. § 112, First Paragraph, Rejections*

The Examiner rejected claims 1-6, 14-15, 20-21, 24-33, 35-39, 41-45, 47, 54, 60-61, 63, and 67-68 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant

art that the inventor(s), at the time of the application was filed, had possession of the claimed invention (a “written description” rejection). In particular, the Examiner asserts that the claims are directed to a genus of nucleic acids with codon and transcriptional regulatory sequence alterations, as well as alterations in the encoded protein up to 15% of the sequence, and that the specification does not teach any representative species of nucleic acid with alterations in the protein sequence that do not alter the function of the protein. The Examiner also rejected claims 1-6, 14-15, 20-21, 24-33, 35-39, 41-45, 47, 60, 61, 63, and 67-68 under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for a variant of a parent DNA molecule encoding a polypeptide identical to a polypeptide encoded by the parent DNA, having more than 25% of the codons altered and having a reduced number of transcription regulatory sequences than the parent nucleic acid or for any nucleic acid which will hybridize to SEQ ID NO:9 under high stringency conditions and encode a polypeptide having luciferase activity, does not reasonably provide enablement for any variant DNA molecule encoding a polypeptide having at least 85% identity to a wild-type polypeptide, having more than 25% of the codons altered and having a reduced number of transcription regulatory sequences than the parent nucleic acid or to any nucleic acid which will hybridize to SEQ ID NO:9 under medium stringency conditions, and encode a polypeptide having at least 85% identity to the polypeptide encoded by SEQ ID NO:9, have more than 25% of the codons altered and have a reduced number of transcription regulatory sequences. These rejections are respectfully traversed.

The specification discloses that a *Renilla* luciferase gene and a yellow-green click beetle luciferase gene (YG#81-6G01) were modified by replacing codons and reducing the number of transcriptional regulatory sequences (Examples 1-3). Codons were generally substituted with mammalian high-usage codons and not with mammalian low-usage or *E. coli* low-usage codons, so that in most cases the substituted codons did not add transcription regulatory sequences (page 53, lines 8-10; page 66, lines 24-26). Two synthetic *Renilla* luciferase sequences (Rluc ver 2 and Rluc final) and 14 synthetic click beetle luciferase sequences (GRver2, GRver3, GRver4, GRver5, GR6, GRver5.1 (i.e., SEQ ID NO:9), RDver2, RDver3, RDver4, RDver5, RD7, RDver5.1, RDver5.2 and RD156-1H9) are disclosed.

In particular, the Examiner is requested to note that one amino acid substitution is present in the protein encoded by the two synthetic *Renilla* luciferase sequences relative to a wild type

*Renilla* luciferase sequence, and a number of amino acid substitutions were introduced to codons in synthetic click beetle luciferase genes relative to a parent luciferase sequence: GRver2-GRver5, and GRver5.1 have 1 amino acid substitution (related to a substitution associated with green light) relative to parent sequence YG#81-6G01; RDver2-RDver5 and RDver5.1 have 4 amino acid substitutions (related to substitutions associated with red light) relative to YG#81-6G01; RDver5.2 has 5 amino acid substitutions (related to substitutions associated with red light and improved spectral properties) relative to YG#81-6G01; and RD156-1H9 has 9 amino acid substitutions (related to substitutions associated with red light, improved spectral properties and improved luminescence intensity) relative to YG#81-6G01 (see Figure 3 for a comparison of the amino acid sequences encoded by the synthetic click beetle luciferase sequences).

Therefore, Applicant has described representative reporter protein sequences with amino acid substitutions relative to a wild type sequence, which representative proteins have reporter activity.

Further, numerous substitutions have been introduced into beetle luciferases without affecting the reporter property of the substitution variants (see, e.g., Kajiyama et al., Protein Engineering, 4:691 (1991)), Wood et al., J. Biolumin., 4:31 (1989), Wood et al., J. Biolumin., 5:107 (1990) and Sala-Newby et al., Biochem. J., 279:727 (1991)) (a copy of each is enclosed herewith) and U.S. Patent No. 5,670,356 (a reference cited against the claims under 35 U.S.C. § 103(a)). Likewise, numerous substitutions have been introduced into other reporter proteins, such as GFP (see U.S. Patent No. 5,874,304, a reference cited against the claims under 35 U.S.C. § 103(a)). Thus, it is well within the skill of the art worker to predictably substitute amino acids in a reporter protein.

Hence, Applicant's specification fully satisfies the requirements of 35 U.S.C. § 112(1).

#### The 35 U.S.C. § 103(a) Rejection

The Examiner rejected claims 1-9, 11-12, 14-15, 20-21, 24-39, 41-45, 60-63, and 67-68 under 35 U.S.C. § 103(a) as being unpatentable over Sherf et al. (U.S. Patent No. 5,670,356) in view of Zolotukhin et al. (U.S. Patent No. 5,874,304) and Iannacone et al. (Plant Mol. Biol., 34:485 (1997)). This rejection is respectfully traversed.

Sherf et al. disclose a synthetic firefly luciferase gene (*luc*<sup>+</sup>) in which 3 internal palindromic sequences, 5 restriction endonuclease sites, 4 glycosylation sites, and 6 transcription factor binding sites were removed, and codons were altered at sequences specified in Table 2 to codons preferred in mammalian cells, relative to a wild type firefly luciferase gene (*luc*). Of the twenty 6 to 30 bp regions which were modified, 6 regions included modifications with a dual purpose, i.e., one region was modified to eliminate a glycosylation site and a transcription factor binding site, three regions were modified to eliminate a transcription factor binding site and improve codon usage, one region was modified to eliminate two transcription factor binding sites (but not improve codon usage), and another region was modified to improve codon usage and eliminate a restriction endonuclease recognition site.

Sherf et al. also disclose that a vector encoding Luc<sup>+</sup> or Luc was introduced to four mammalian cell lines. NIH3T3 and HeLa cells transfected with *luc*<sup>+</sup> DNA had significantly higher levels of luciferase activity relative to NIH3T3 and HeLa cells transfected with *luc* DNA (Table 3), while CHO and CV-1 cells transfected with *luc*<sup>+</sup> or *luc* DNA had comparable luciferase activity. However, it is unclear what alterations in *luc*<sup>+</sup> DNA increased luciferase activity in mammalian cells, and why those alterations did not uniformly increase luciferase activity in all the tested mammalian cells. In contrast, a synthetic *Renilla* luciferase gene of the invention was expressed at significantly higher levels relative to a wild type *Renilla* luciferase gene in NIH3T3, HeLa, CHO and CV-1 cells (Table 10).

Sherf et al. do not teach or suggest a synthetic nucleic acid molecule having a codon composition which differs at more than 25% of the codons relative to those of a corresponding wild type nucleic acid sequence, which codons are selected so as to result in the synthetic nucleic acid molecule having a reduced number of a combination of transcription factor binding sites, and intron splice sites, poly(A) sites and/or promoter sequences.

A humanized version of a green fluorescent protein (GFP) gene is disclosed in Zolotukhin et al. in which 88/238 of the codons in the gene were altered (column 13, lines 1-4).

Zolotukhin et al. do not disclose or suggest a synthetic nucleic acid molecule which has a codon composition which differs at more than 25% of the codons relative to those of a corresponding wild type nucleic acid sequence, which codons are selected so as to result in the

synthetic nucleic acid molecule having a reduced number of a combination of transcription factor binding sequences, and intron splice sites, poly(A) sites and/or promoter sequences.

Iannacone et al. disclose synthetic *Bacillus thuringiensis* Bt43 genes (abstract) which encode an insect toxin. To prepare those genes, Iannacone et al. modified the nucleotide sequence of Bt43 in four target regions to avoid sequences which might destabilize mRNA, sequences such as ATTTA sequences, polyA sequences, splicing sites and A or T strings > 4, and to improve codon usage for plant expression (abstract and page 490). However, splicing sites were apparently not removed (Table 1). Five constructs, one with the wild type Bt43 gene and four with synthetic Bt43 genes, i.e., BtE, BtF, BtH and BtI (see Figure 2 and Table 1), were introduced to eggplant or *Solanum integrifolium* cultures, and transgenic plants regenerated. Bt43-specific polyA+ RNA in the plants was detected by Northern blot analysis (Figure 4).

It is disclosed in Iannacone et al. that no Bt43-specific bands were detected in lanes for plants with the wild type Bt43 gene even after long exposures (page 491). Interestingly, plants expressing the BtE gene had higher levels of Bt43-specific polyA+ mRNA than plants expressing the BtF gene, a gene which had fewer (A)>4 and (T)>4 strings, one less ATTTA sequence, and more codons modified relative to the BtE gene. In fact, no full size Bt43-specific mRNA was detected in BtF transgenic plants in contrast to BtE transgenic plants (page 494). Thus, the additional modifications in BtF relative to BtE, i.e., additional codon substitutions, and a reduced number of ATTTA sequences and A or T strings > 4, reduced full length mRNA.

And although full length BtE polyA+ RNA was present in BtE transgenic plants, no Bt toxin was produced, leading the authors to conclude that a 1.2 Kb unmodified domain in the BtE gene is a major candidate for translational blockade (page 494).

The authors of Iannacone et al. conclude that the increased level of Bt43-specific mRNA in BtE and BtF transgenic plants compared to wild type Bt43 transgenic plants could be related to the elimination of destabilizing sequences and that the AUUUA string in wild type Bt43 is a major candidate for the instability and untranslatability of Bt43 mRNA (page 494).

Iannacone et al. do not disclose or suggest a synthetic nucleic acid molecule which encodes a reporter polypeptide having a codon composition which differs at more than 25% of the codons relative to those of a corresponding wild type nucleic acid sequence, which codons are selected so as to result in the synthetic nucleic acid molecule having a reduced number of a

combination of transcription factor binding sequences, and poly(A) sites, intron splice sites and/or promoter sequences.

The Examiner asserts that the combined disclosures of the cited references clearly suggest that alterations of most of the codons of genes which are to be expressed in evolutionarily highly distinct organisms from those in which they evolved substantially improve the levels of expression in the new host, and that one of skill in the art would have had a reasonable expectation of success in view of the results of Zolotukhin et al. and Iannaccone et al. who each successfully improved the expression of such a gene by such a modification.

The combination of references does not disclose or suggest Applicant's invention as each reference discloses a different way to modify the coding sequence of a gene to increase expression, i.e., Zolotukhin et al. disclose codon modification alone generally throughout a green fluorescent protein gene, Sherf et al. disclose limited and targeted modification (modifications in 20 regions of 6 to 30 bp) of a firefly luciferase sequence to introduce or remove cloning sites, alter insect codons to mammalian codons, and to remove post-translation modification sites, secondary structure, and transcription factor binding sites, and Iannaccone et al. disclose targeted modification of four regions of a toxin gene to alter *Bacillus* codons to plant codons, and to remove polyA sequences, ATTAA sequences and strings of A or T > 4.

There is no teaching or suggestion in any of the references, or the combination of the references, to modify a nucleic acid sequence by altering more than 25% of the codons of a corresponding wild type nucleic acid sequence, which codons are selected so as to result in the synthetic nucleic acid molecule having a reduced number of a combination of transcription factor binding sequences, and intron splice sites, poly(A) addition sites and/or promoter sequences. Moreover, there is no teaching in the cited art that codon modifications can introduce undesirable sequences into the synthetic gene and that codon selection can decrease the introduction of those sequences.

In addition, if altering codon composition in an open reading frame to codons preferred in a heterologous host alone increases expression in a heterologous host, then there would be no motivation for the art worker to make any other changes, e.g., those which may reduce aberrant transcription. Nor does any of the cited art point the art worker to which changes in combination would be useful in that regard.

Further, one of ordinary skill in the art in possession of the cited art would have no reasonable expectation that any particular set of changes would improve activity in a gene that is to be expressed in a highly evolutionarily distinct cell. For example, codon alterations and a reduction in polyA sequences, strings of A or T > 4 and ATTTA sequences in a Bt43 sequence yielded mRNA but did not yield a detectable protein (BtE in Iannaccone et al.), and further codon alterations and a further reduction in strings of A or T > 4 and ATTTA sequences did not yield detectable full length mRNA (BtF in Iannaccone et al.). Thus, an increase in codon substitutions and a decrease in RNA destabilization sequences in a synthetic gene do not necessarily improve the transcriptional characteristics of the synthetic gene relative to the reference gene. In addition, it is unclear what changes to the Bt43 or *luc* sequence resulted in improved activity in a heterologous host and why replacement of codons in *luc* with codons preferred in mammals and other alterations which resulted in *luc*<sup>+</sup> did not improve luciferase activity in all mammalian cells which expressed Luc<sup>+</sup>.

Therefore, it is unexpected that a synthetic gene of the invention would have reduced aberrant transcription relative to a corresponding wild type sequence

Accordingly, withdrawal of the § 103 rejection is respectfully requested.

**AMENDMENT AND RESPONSE UNDER 37 CFR § 1.116 – EXPEDITED PROCEDURE**

Serial Number: 09/645706

Filing Date: August 24, 2000

Title: SYNTHETIC NUCLEIC ACID MOLECULE COMPOSITIONS AND METHODS OF PREPARATION

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Dkt: 341.005US1

**CONCLUSION**

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612) 373-6959 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

KEITH V. WOOD ET AL.

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.  
P.O. Box 2938  
Minneapolis, MN 55402  
(612) 373-6959

Date

April 6, 2004

By Janet E. Embretson  
Janet E. Embretson  
Reg. No. 39,665

**CERTIFICATE UNDER 37 CFR 1.8:** The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first-class mail, in an envelope addressed to: Mail Stop AF, Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 6<sup>th</sup> day of April, 2004.

Name

Dawn M. Rose

Signature

Dawn M. Rose